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ROY WALDO MINER

DROP DENSITY, WEIGHT, VOLUME, AND TIME VARIATIONS DURING CHROMATOGRAPHIC COLUMN DEVELOPMENT

BY

N. A. SOLOMON AND S. Z. LEWIN

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Analysis of the effluent collected in the course of column chroma- graphy is the most difficult and tedious part of the entire chromato- aphic technique, and a number of ingenious and complex instruments ive been devised to perform this function continuously and automati- lly. In the absence of a continuous record of effluent composition, bitrary fraction-cutting procedures must be used. It is evident, however, at rational fraction-cutting, based upon the composition of the effluent, greatly to be preferred. Many of the properties of the effluent have een utilized in efforts to devise rational procedures of fraction col- ction. Chief among those which have been more or less successfully ployed are refractive index, absorption spectra, fluorescence, re- ectance, dielectric constant, conductance, and radioactivity. However, anges in the density, surface tension, viscosity and electrokinetic operties of the effluent during elution have not as yet been exploited. he present investigation was undertaken to provide some insight into e nature and magnitude of the variations in the latter properties during everal types of chromatographic development, and to explore the ossibilities inherent in these properties for application to the problem f rational fraction cutting.

Experimental

In most of the experiments, the chromatographic column was 15 cm. ong and 1 cm. in inside diameter. The bottom of the column rested on a itted glass disc having pores 40 to 60 microns in diameter and covered ith a circle of filter paper. After passing through the disc, the effluent lowed through a capillary tube 5 cm. long and 0.8 mm. in inside diameter efore issuing as droplets from the tip of the capillary. The space between e underside of the fritted disc and the top of the capillary tube was illed with small glass beads to eliminate the incorporation of air bubbles n the effluent. This arrangement was found to give reproducible results ith a convenient time interval between effluent drops.

After the packed column had been washed with solvent for several ours, the mixture to be resolved was placed on the column, and de- eloped. The hydrostatic head of fluid was maintained constant by eans of a constant-level feeding device. The drops issuing from the olumn were collected individually in tared vessels and weighed on a microbalance. The densities of the individual drops were determined by the falling-drop technique[1] in a *m*-xylene-bromobenzene mixture. Drop volumes were calculated from the weights and densities. The time intervals elapsing between the detachment of successive drops from the capillary tip (hereafter called the drop times) were measured by means of an electric stop clock reading to 0.01 seconds and controlled by a telegraph-type tapping key. Appropriate sensitive color reactions were applied to the drops to test for the presence of solutes. The collection and timing of drops was commenced when the adsorbed material approached close to the bottom of the adsorbent column.

The systems studied in most of the experiments were (1) cobalt (II), copper (II), and iron (III) nitrates and chlorides adsorbed on alumina and eluted by water, 0.1 M Rochelle salt solution, and 0.3 M sodium

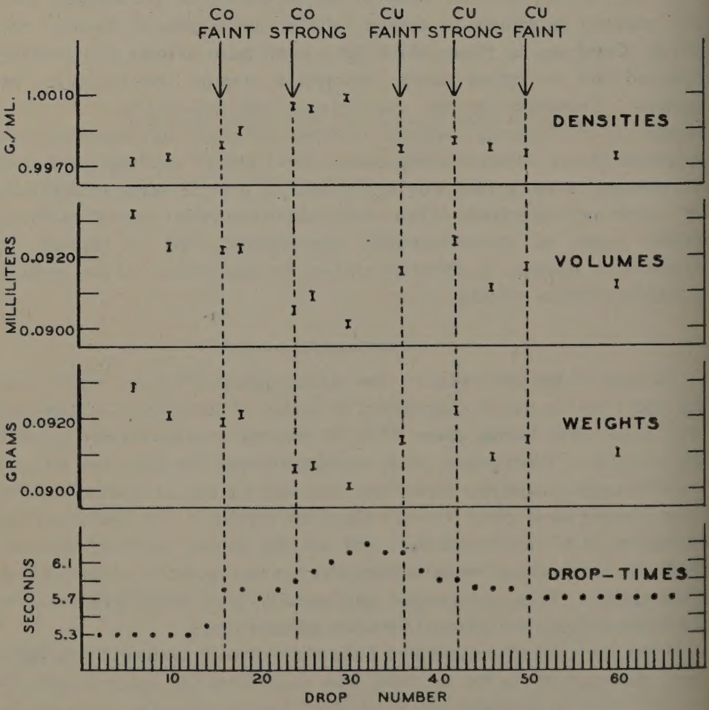

FIGURE 1. Elution chromatogram of 0.1 ml. of a solution 6.67 per cent in cobalt (II), copper (II) and iron (III) nitrates on acid-washed alumina. The iron and a portion of the copper could not be eluted from the column.

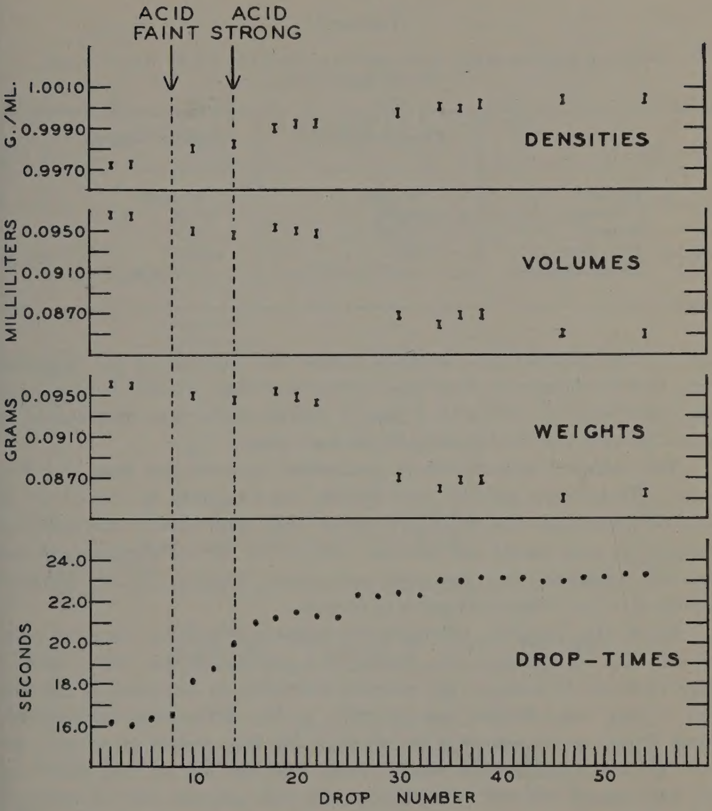

FIGURE 2. Frontal analysis of 0.5 per cent acetic acid plus 0.5 per cent propionic acid in water on mixed alumina charcoal.

chloride solutions; (2) acetic and propionic acid mixtures in water developed by frontal analysis on mixed alumina-charcoal adsorbent.

More than a score of experiments of types No. 1 and No. 2 were performed and, in every case, the following relationships were observed. When the first solute began to break through the column, the drop density showed an increase, drop weight and volume decreased, and drop time increased. When the second solute began to break through, the drop densities decreased somewhat in the elution analyses, and increased in the frontal analyses. In all cases, however, the changes in density were

TABLE 1

COMPARISON OF MOST CONCENTRATED EFFLUENT DROP WITH PURE SOLVENT

	Elution analysis	*Frontal analysis*
Density	+0.35%	+0.35%
Volume	−3.3%	−11%
Weight	−3.3%	−11%
Drop time	+18%	+50%
Concentration	0.5%(w./v.)	1.0%(w./v.)

accompanied by changes in drop weight and volume of the opposite sign, and by changes in drop time of the same sign. These relationships are illustrated in FIGURES 1 and 2, which show data obtained in a typical elution and frontal analysis respectively.

The relative differences in properties between the most concentrated effluent drop and the pure solvent are tabulated in TABLE 1. It will be noted that the density changes were quite small, the relative changes in drop weight and volume were 10- to 30-fold greater, and the drop-time changes were the most pronounced, namely, 50- to 150-fold greater than the relative changes in density.

Since the relative difference in refractive indices between pure solvent and an effluent drop having the concentrations shown in the table is about the same as the relative difference in densities, it follows that a very considerable magnification of the differences between effluent drops can be achieved by following the drop weight or volume. An even greater magnification would result from the use of drop times as the indicator of effluent composition, and this property should prove to be particularly convenient and effective in routine chromatographic work.

In substantiation of this conclusion, we have applied the drop-time technique to a number of elution analyses of dye mixtures on alumina with aqueous acetone. A tvpical drop time profile is shown in FIGURE 3. The variations are quite large, and occur synchronously with the changes in the composition of the effluent.

In order to shed some light on the role played by the adsorbent in the drop-time variations, several chromatograms were run with a 250-mesh carborundum (silicon carbide) powder as the column material. The drop-time profiles obtained with carborundum and alumina columns are contrasted in FIGURE 3 for an elution of cobalt (II) nitrate, and for a frontal analysis of an acetic-propionic acid mixture.

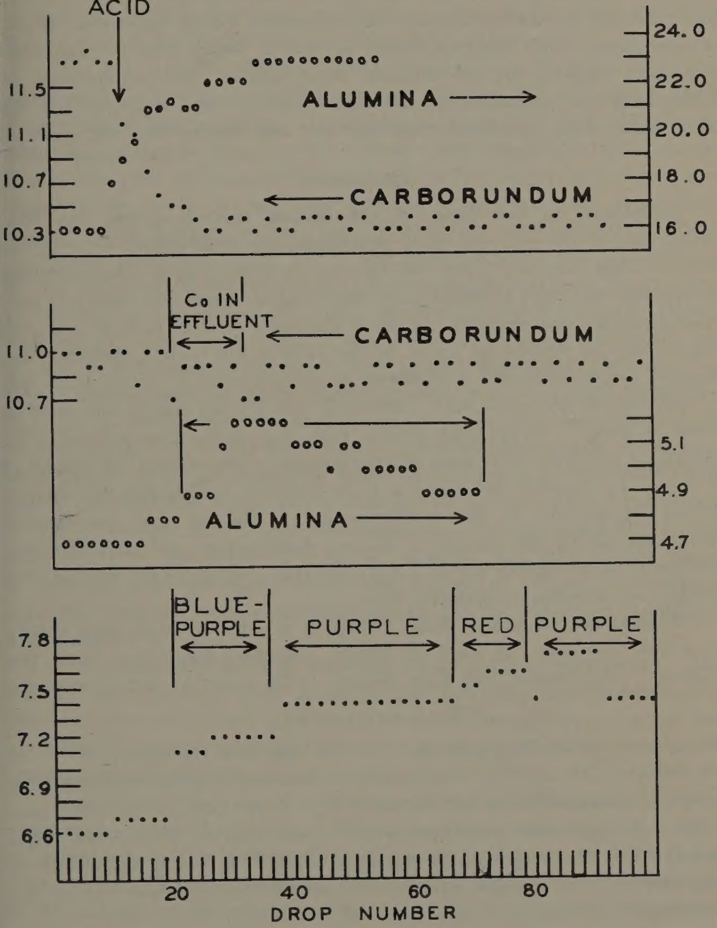

FIGURE 3. Drop-time profiles for several systems:

Top. Carborundum and alumina columns contrasted in frontal analysis of 0.5 per cent acetic acid + 0.5 per cent propionic acid in water.

Middle. Elution of 0.1 ml. of 20 per cent cobalt (II) nitrate in water.

Bottom. Elution of 0.1 ml. of 0.5 per cent gentian violet + 0.5 per cent safranine on alumina with 50 per cent acetone water.

The elutions carried out with 0.3 M NaCl as the solvent showed much smaller relative changes in all properties during the course of the chromatogram. This suggests that the NaCl, which was in high concentration relative to the solutes being eluted, was predominant in determining the properties of the effluent drops, and swamped out the effects that were prominent when this salt was absent from the solvent.

Discussion

Densities. In the case of the elution chromatograms, the concentrations in the effluent drops containing the most solute were of the order of magnitude of 0.5 per cent (w./v.), or about 0.03 M. The densities of aqueous solutions of copper and cobalt nitrates and chlorides of these concentrations are reported[2] to be of the order of 0.25 to 0.30 per cent larger than the density of water. In the case of the frontal analyses, the effluent drops contained a maximum of 0.5 per cent of acetic acid and 0.5 per cent of propionic acid. The density of 1.0 per cent acetic acid in water is about 0.16 per cent greater than that of the pure solvent. The agreement between these data and the present observations, as indicated in FIGURES 1 and 2 and TABLE 1, is probably well within the experimental uncertainty in the measurements. It follows that the density variations during the course of column development are so small that the use of this property as the key to fraction cutting would require very precise and sensitive instrumentation.

Weights and volumes. When a drop is allowed to grow very slowly at a flat capillary tip until it finally detaches itself and falls, the ("ideal") drop weight has been shown[3] to be given by the expression:

$$W = 2\pi r \gamma f(r/V^{1/3}) \qquad (1)$$

where γ is the surface tension, r is the capillary tip radius, V is the drop volume, and $f(r/V^{1/3})$ is an empirical function. The latter function is relatively insensitive to variations in V; a 1 per cent change in V produces a 0.33 per cent variation in $r/V^{1/3}$, and at $r/V^{1/3} = 1.0$, this corresponds to a change of only 0.1 per cent in $f(r/V^{1/3})$. Hence, variations in the "ideal" drop weight will be due principally to variations in the surface tension.

A 0.5 per cent solution of $CuSO_4$ in water has a surface tension that is approximately 0.1 per cent greater than pure water, and a similar slight increase in surface tension is characteristic of dilute solutions of all strong electrolytes in water.[4] On the other hand, the aliphatic acids are capillary-active, and reduce the surface tension of water. A 0.5 per cent solution of acetic acid has a surface tension that is 2.3 per cent lower than that of water. 0.5 per cent propionic acid causes a decrease of 6.8 per cent in the surface tension.[5] A solution that is 0.5 per cent in both acetic and propionic acids may be expected to have a surface tension that is 8 to 9 per cent less than that of pure water.

The drop weights obtained in the elutions were as much as 3.3 per cent less than the drop weight for water, whereas if these were "ideal" drop weights, the preceding considerations show that they should have been approximately equal to the "ideal" drop weight for water. In the frontal analyses, the drop weights were up to 11 per cent less than that of water, which is to be compared with the 8 to 9 per cent decrease in "ideal" drop weight to be expected from the surface active solute.

These relatively large deviations from the ideal are probably due to the short drop times employed (5 to 6 seconds for the elutions, 16 to 24 seconds for the frontal analyses); for Harkins and Brown have shown[3] that EQUATION (1) is strictly applicable only when the drops form slowly (3 to 6 minutes per drop). With shorter drop times, the amount of liquid flowing into the drop, during the time it is detaching itself from the tip, is enough to increase its weight appreciably over that of the "ideal" drop. Thus, the drop-weight variations observed in the present work appear to be determined by these deviations from the "ideal" drop due to the time variations, rather than by the surface tension variations.

It is evident that, under the conditions of these experiments, with drop times of the order of 5 to 25 seconds, the drop weight shows relatively large variations during the course of column development, and this property offers definite promise as an indicator of effluent composition.

The variations in drop volumes were found to parallel the drop weights very closely. This is due to the fact that the changes in drop density were very small relative to the changes in drop weights. The preceding considerations and conclusions apply, therefore, also to the interpretation of the drop volume relationships.

Drop times. The time elapsing between the fall of successive drops must depend upon (1) the rate at which liquid percolates through the bed of adsorbent and its fritted glass support; (2) the rate at which liquid flows through the exit capillary; and (3) the volume attained by the drop at the moment of detachment. If the resistance to fluid flow offered by the adsorbent bed and fritted disc were small compared to that offered by the exit capillary, the variations in drop time should be calculable from Poiseuille's equation:

$$\eta = \frac{\pi g p R^4 t}{8 V l} \qquad (2)$$

where R is the radius of the capillary, p is the (constant) pressure head, and t is taken as the time necessary for the drop to grow to its maximum volume, V. This gives:

$$\frac{dt}{t} = \frac{dV}{V} + \frac{d\eta}{\eta} \tag{3}$$

If correction is made for kinetic energy and end effects, as in the Wilberforce equation[6]:

$$\eta = \frac{\pi g p R^4 t}{8 V 1} - \frac{m \rho V}{8 \pi 1 t} \tag{2'}$$

where m is a constant, the relationship between drop time, volume and viscosity variations becomes:

$$\frac{dt}{t} = \frac{dV}{V} + \frac{d\eta}{\eta + \frac{m \rho V}{4 \pi 1 t}} \tag{3'}$$

The rate of flow of fluids through porous beds has been treated by Darcy,[7] Kozeny[8] and others, and, in the absence of electrokinetic effects, is governed by a relationship of the form:

$$\eta = \frac{K A p t}{V 1} \tag{4}$$

which also leads to EQUATION No. 3, above, for the variation of drop times with viscosity variations.

The viscosities of 0.5 per cent solutions of the salts and acids used in these experiments are 1 to 2 per cent greater than that of water.[9] Since the largest variations in drop volumes were –3.3 per cent and –11 per cent for the elutions and frontal analyses respectively (TABLE 1), the preceding considerations [EQUATIONS Nos. 3 or 3′] would predict for the maximum variations in drop time about –2 per cent and –10 per cent. This is in marked disagreement with the +18 per cent and +50 per cent variations actually observed with the alumina columns, although it is in good agreement with the results obtained with the carborundum columns.

Thus, it follows that, with alumina as the adsorbent, the drop-time variations are not simply related to the viscous flow of the liquid through the column or the capillary tube. The interpretation of the present data for alumina columns must be sought in some other factor.

The data illustrated in FIGURES 1 and 2 show that the changes in drop time occurred in close synchronism with changes in the composition of the effluent. The controlling factor, therefore, must have been the rate of flow of fluid through the base of the alumina bed, or the fritted glass disc that supported the column, and this flow rate must be very sensitive to changes of concentration and composition of relatively dilute solutions. This suggests that the factor that is responsible for the drop-time variations is the streaming potential established between the stationary pore walls and the flowing electrolyte solution.

Although specific data on streaming potentials for the systems involved in the present work are not available, the general effects reported for other systems[10] indicate that variations in the zeta potential existing at the pore-solution interfaces which could reasonably be produced in the present instances would account completely for the drop-time variations. For example, while the cobalt or copper salt is on the column, the streaming potential would be close to zero in those regions where the electrolyte concentration is high. As the electrolyte passes out of the base of the column, the streaming potential in the pores at the base of the column would increase (in absolute magnitude), the rate of flow of liquid would correspondingly decrease, and the drop times would tend to rise. However, the streaming potentials existing in the fritted-glass disc would simultaneously change in the opposite sense to the changes occurring at the base of the adsorbent bed, since, in the frit, the electrolyte concentration is increasing while it is decreasing in the bed. The observed changes in drop time would be the resultant of these opposite effects in the alumina and in the glass pores. Another factor that must be involved is the presence of a low, but finite, concentration of ions in the effluent due to the dissolution of the alumina, and the role that may be played by these ions in affecting the zeta potential of the glass frit when solvent is passing through it.

The probability that streaming potentials, rather than changes in viscosity, volume, or other property characteristic of the liquid phase alone, lie at the root of the drop-time variations is shown by the entirely different kind of drop-time variations observed with carborundum columns as contrasted with alumina columns (FIGURE 3). The silicon carbide is a much poorer adsorbent than is alumina, and would be expected to show very little electrokinetic activity. In substantiation of this, the drop-time variations with the carborundum columns are very close to the predicted effects based on the fluid flow equations.

Although a more detailed interpretation of the drop-time variations must await further studies of the phenomena involved, the present work clearly demonstrates that the drop time offers great promise as a new and potentially very useful tool in the development of rational fraction-cutting procedures for column chromatography with colorless solutes.

Acknowledgment

The existence of variations in drop time that reflect the composition

of the effluent was, to our knowledge, first recognized by R. H. Müller. In 1950, Müller proposed to one of us (N.A.S.) that automatic instrumentation for chromatography might be successfully based upon this effect. The present investigation was undertaken to determine the validity of Müller's observation, and to lay a groundwork for subsequent instrumental approaches.

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